Variable shrimp in variable environments: reproductive investment within *Palaemon varians*

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ABSTRACT: The maternal environment may influence the quantity and quality of resources invested in offspring (per-offspring provisioning) and this trait, in turn, affects larval fitness and may carry-over into early juvenile life. Here, per-offspring investment was measured across three consecutive breeding seasons for the caridean shrimp, *Palaemon varians*. Egg and hatchling larval dry weight as well as hatchling larval biochemical composition were measured. Results indicate that egg volume is positively correlated with dry weight, but egg volume as a proxy for dry weight should be used with caution. Correlations were identified between per-offspring investment and average breeding season temperature, but the period over which temperature was averaged was important to whether these correlations were positive or negative, highlighting the complexity of identifying environmental influence on phenotypic traits. The most important factor determining offspring size and per-offspring investment was maternal size, which may be influenced by the environment as carapace length varied significantly between breeding seasons. These data demonstrate variation in egg and larval composition, related to maternal size, which will affect early life traits and survivorship for *P. varians*.

KEY WORDS: Per-offspring investment · Maternal size · Egg volume · Egg dry weight

1. INTRODUCTION

Salt marshes are intermediate coastal habitats at the confluence of terrestrial, marine, and freshwater environments. Like intertidal shores, the flora and fauna inhabiting salt marsh ecosystems are evolved to cope with variations in temperature, salinity, and oxygen concentrations; which occur as the influence of the marine environment ebbs and flows with the tides. The variable shrimp*, Palaemon varians* Leach, 1814 (formerly *Palaemonetes varians*; De Graves & Ashelby 2013) (also known as common ditch shrimp or Atlantic ditch shrimp), is a common estuarine and brackish-water invertebrate on North Western European coasts in the NE Atlantic. *Palaemon* spp are present in marine, brackish-, and fresh-water environments and the evolutionary transition from the ancestral marine habitat to freshwater has occurred many times within the palaemonine generally, with species exhibiting a spectrum of adaptations to increasingly freshwater environments (e.g. Anger 2001, Oliphant & Thatje 2013). Characteristic adaptations to increasingly brackish and freshwater environments include producing larger yolky eggs and an increasingly abbreviated larval phase, or direct development (Anger 2001). Within *Palaemon* of the British Isles, the marine species *P. serratus* (Pennant, 1777) and *P. elegans* Rathke, 1836 develop through ~8 and 6-9 larval instars, respectively, whilst the brackish-water *P. varians* develops through 4-5 larval instars (Fincham 1977, 1979, 1983, Oliphant et al. 2013). *P. varians* larvae are thought to be exported from the brackish water environment into the ancestral marine environment for larval development and recolonise brackish-water during juvenile stages (Fincham 1979, Oliphant & Thatje 2014). Whilst predation risk is likely greater, the prevalence of food resources and the proximity of the peripheral brackish-water to marine are likely important factors in larval export strategy (Anger 2001). *P. varians* eggs are loaded with greater provisions than other marine species in the genera, and the larvae can be considered facultative planktotrophs through the first larval instar, during which time they can presumably be exported to the marine environment (Oliphant & Thatje 2014). Whilst the effects of per-offspring investment on larval traits have been studied (see below), a study of this trait in a wild palaemonid population, and factors that influence it, has to our knowledge, not been published.

For marine invertebrates, evidence suggests that environmental conditions during oogenesis influence egg quality (e.g. Bayne et al. 1978, Chester 1996, George 1990, Qian and Chia 1991, Qian 1994). For arthropods, the influence of temperature on offspring size has been demonstrated repeatedly and there are numerous examples of temporal, seasonal changes and geographic differences in per-offspring investment (e.g. Fischer et al. 2003a,b,c, Bas et al. 2007, Fischer et al. 2009, Liefting et al. 2010, González-Ortegón et al. 2018, Lardies & Castilla 2001, Lardies & Wehrtmann 2001, Fischer et al. 2009, Urzúa et al. 2012, González-Ortegón et al. 2018). For the brown shrimp, *Crangon crangon* (Linnaeus, 1758), eggs with higher per-offspring investment are produced in cooler winter months (Urzúa et al. 2012, Urzúa & Anger 2013). This shift in per-offspring investment influences larval development with larvae from winter eggs developing through fewer larval instars than those from summer eggs (Linkt 1995; see Giménez 2006). The influence of per-offspring investment on larval development and survivorship has been demonstrated for a number of decapod species, including *P. varians* (e.g. Giménez and Torres 2002, Giménez et al. 2004, Giménez 2006, Oliphant and Thatje 2013, Oliphant et al 2014, González-Ortegón and Giménez 2014). The larval development of *P. varians* is well characterised and recently it has become an important study species for experimentation on decapod physiology and responses to hyperbaric pressure (e.g. Oliphant et al. 2011, Morris et al. 2015, Brown et al. 2017, Peruzza et al. 2018). These include studies on temperature tolerance, though detailed data on native temperature range and salinity are rarely published. Here, we assess variations in per-offspring investment across three breeding seasons for a population of *Palaemon varians* from Lymington saltmarsh, Southern England. Further we report (as supplementary data) temperature and salinity data from a three year monitoring effort for this population. We hypothesis that maternal per-offspring investment will be determined by female size and by environmental temperature; such that larger, better provisioned offspring will be produced by larger females and under cooler conditions.

2. MATERIALS & METHODS

2.1 Collection site

Palaemon varians were collected from Lymington salt marshes (Hampshire, England, UK). This area of salt marsh comprises numerous inter-connected lagoons and drainage ditches and is surrounded by a seawall in which numerous sluice gates open out into the West Solent. The collection site was a ~100 m section of drainage ditch ~0.5 m deep and which varied in width from ~1 to 3 m (WGS84: 50.740141°N, 1.537707°W; GEOREF: MKPF27784438). Temperature data loggers (nke instrumentation S2T600 Temperature data logger, Hennebont, France) were deployed at three sites along the ditch from April 2011 until August 2013; temperature data were logged every 30 minutes. The site was visited monthly over three years (April 2011 to August 2013) so that temperature data could be downloaded to a laptop and water samples taken from adjacent to temperature data loggers during these visits. Water samples were transported to the National Oceanography Centre Southampton (NOCS) and their salinity measured using a Hach HQ30d portable conductivity meter. In addition, air temperature data and rainfall data were downloaded from http://www.southamptonweather.co.uk/; accessed from October 2012 to August 2013.

2.2 Collections and maintenance of *Palaemon varians*

Adult *Palaemon varians* were collected by hand netting (6 mm mesh size) and placed in 10 l buckets containing water from the point of collection. These buckets were sealed with water-tight lids and transferred (<1 hr) to the research aquarium at the NOCS. Here, aeration was provided whilst shrimp were processed. In year 1 (2011) approximately 150 *P. varians* were sampled monthly between February and July 2011 for population size frequency analysis. Ovigerous females (those brooding eggs attached to the pleopods), were separated out and all other shrimp caught were preserved in 4 % formalin. In years 2 and 3 (2012, 2013), only ovigerous females were collected during the breeding season (April-July) for sampling of newly extruded eggs and newly hatching larvae. Males and non-ovigerous females were returned immediately to the ditch. At the NOCS, the development of broods was assessed under a stereo dissection microscope (in accordance with Müller et al. 2004). Females with newly extruded eggs (stage I and II, *sensu* Müller et al. 2004) were blotted dry, placed in zip-lock plastic bags and frozen at -80 °C. Ovigerous females with late stage embryos (stages VII and VIII, *sensu* Müller et al. 2004) were maintained individually in 1 l plastic buckets, filled with ~850 ml of 1 µm-filtered, 32 salinity seawater, in incubators set to the field temperature at the time of collection and 12:12 light:dark cycle. Shrimp were checked every day (am) for hatched larvae and only larvae hatching within 1-3 days of collection were sampled.

2.3 Size frequency analysis

Shrimp were sexed (via the presence/absence of the appendix masculine) and the post-orbital carapace length was measured using digital venier calipers (accuracy 0.01 mm). Post-orbital carapace length data for ovigerous females, retained for assessment of per-offspring investment, were also used in size frequency analysis.

2.4 Assessment of per-offspring investment

Ovigerous *P. varians* with newly extruded eggs were defrosted individually, and a sample of 15 eggs dissected from the 1st right pleopod. A second assessment of egg development was made before eggs were individually blotted dry and transferred to pre-weighed tin capsules and freeze-dried for 24 hr and weighed for dry weight (DW, µg). For ovigerous *P. varians* with late stage embryos, upon hatching, a sample of 15 newly hatched, actively swimming larvae per female were separated from mothers using a plastic pipette, blotted dry and transferred to pre-weighed tin capsules, frozen at -80 °C, and later freeze-dried for 24 hr and weighed for DW. For $n = 5$ larvae per female hatching in May and June of 2012, carbon and nitrogen content were measured using a CHNS-O EA1108-elemental analyser (Carlo ERBA Instruments) and a chitin standard. The post-orbital carapace length was measured for all females; those carrying stage I and II eggs, and those from which larvae were sampled. A measure for maternal investment per offspring was calculated by dividing offspring dry weight by maternal post-orbital carapace length (µg/mm).

2.5 Egg volume measurements and calculations

Ovigerous females with newly extruded eggs, collected during the 2011 breeding season were treated as above. After the second assessment of development, a photograph of each egg was taken ($n = 15$ per female). Egg-length and -width were measured from photographs using SigmaScan V6 software, which calculated egg 'major axis' and 'minor axis' (length and width, respectively). Egg volume was calculated using the equation for the volume of a prolate spheroid; Volume = $^{4}/_{3}\,\pi\, a^2 b$ (where $a = {}^{1}/_{2}$ minor axis and $b = {}^{1}/_{2}$ major axis).

2.6 Per-offspring investment and environmental temperature

Correlation analysis was used to assess the relationship between per-offspring investment (either offspring dry weight or maternal investment per offspring) and average temperature, average daily temperature minimum, and average daily temperature maximum, averaged from the start and end of the winter cold period until collection date. The winter cold period was defined as the period from when temperature first dropped below 5 °C until temperature rose and remained above 5°C. In addition, growing degree days,0 (GDD,0) were calculated across the breeding season starting from the winter cold period. GDD,0 was calculated using the formula GDD=((T_{max} + T_{min})/2)-T_{base},0 in which T_{max} = maximum temperature, T_{min} = minimum temperature, and T_{base} = threshold temperature. T_{base} was set to 5°C as this is thought to be the threshold temperature above which gonad maturation can initiate in *P. varians* (Hindley 2001). If average day temperature, (T_{max} + T_{min})/2, was less than the threshold temperature, T_{base} (5°C), the resulting GDD was given as 0 (hence GDD,0). Correlation analysis was used to assess if there was a relationship between GDD,0 and maternal investment per offspring.

2.7 Statistical analysis

Data were tested for normality of distribution and equality of variance using Kolmogorov-Smirnov Test and Levene's Test, respectively. Analysis of variance of maternal post-orbital carapace lengths between months and between years was done by General Linear Model (GLM) ANOVA. For females with ovigerous stage I-II eggs, only n = 1 were collected each year in July and low numbers were collected throughout the breeding season of 2013 (n = 9; Figure 2); therefore, these data were not used for statistical analysis but are presented in Figure 2A for completeness. For correlation analysis, relationships between factors were tested by Spearman's rank correlation.

3. RESULTS

3.1 Size frequency

Palaemon varians size frequency was measured from February until July 2011 (Figure 1). Throughout this period, the percentage of males collected increased from 42.9 % in February to the highest value of 74.8 % in June (Figure 1A). Ovigerous females were first sampled in April, carrying stage I-II eggs, and representing 36.4% of females sampled. The percentage of females carrying stage I-II eggs sampled decreased through May (33.3 %), June (20.8 %), and July (2.1 %) to 0.0 % in August. Ovigerous females carrying stage VII-VIII embryos were first sampled in May (11.1 % of females) and this increased in June (26.4 %) and July (37.5 %), but was 0.0 % in August.

Pre-orbital carapace lengths of *P. varians* sampled ranged from 3.6 to 10.3 mm (Figure 1). Female carapace length ranged between 3.6 and 10.3 mm whilst males were generally limited to smaller size classes, with carapace lengths between 3.9 and 7.8 mm. Carapace length for females with stage I and II eggs were between 4.4 and 8.9 mm, whilst for females with stage VII and VIII they ranged from 6.6 to 9.8 mm. In all months, females were larger than males. Density plots generally form two peaks for males and two, possibly three, for females, suggesting several cohorts for each sex (Figure 1). These data require additional sampling to confirm cohort number and size. In particular, the relatively low number of females sampled is problematic.

3.2 Maternal size

For ovigerous females carrying stage I-II eggs, maternal carapace length varied significantly between months (F = 8.31, $P = 0.001$) and between years (2011-’12; F = 31.24, $P < 0.001$), but there was no interaction between month and year (F = 1.85, $P = 0.166$), indicating that patterns were consistent between years (2011 & 2012; Figure 2A). In both years, females were smaller in May than in April and June. Maternal carapace length of ovigerous females with stage VII-VIII embryos varied between months (F = 8.81, $P < 0.001$) and years (2011-'13; F = 4.52, $P = 0.014$), but there was no interaction between month and year (F = 1.35, $P = 0.260$); females were smaller in June than in May and July across all years (2011-'13; Figure 2B). These data present a consistent trend of smaller females breeding in the middle of the breeding season than at the beginning and end.

3.3 Per-offspring investment

In all years, ovigerous *P. varians* with stage I-II eggs were present in April collections, whilst ovigerous *P. varians* with stage VII-VIII embryos were absent. Similarly, those with stage I-II eggs were rare in July, whilst shrimp with stage VII-VIII were present (Figure 2). Ovigerous shrimp were absent from March and August collections, suggesting that breeding occurs from April to early August.

3.3.1 Relationship between egg volume and dry weight

Egg volume ranged from 0.178 to 0.334 mm^3 with a mean of 0.274 ± 0.024 mm^3 whilst egg dry weight ranged from 117 to 197 µg with a mean of 160.44 ± 14.06 µg. Egg volume was found to correlate to egg dry weight in a strong and positive trend; Spearman's rank correlation rho = 0.635, $p < 2.2e\text{-}16$ (Figure 3).

3.3.2 Relationship between larval dry weight and elemental composition

Hatchling dry weight ranged from 101 to 172 µg with a mean of 133.31 ± 13.99 µg. Hatchling carbon content (%C) ranged from 36.16 to 54.39 % with a mean of 45.91 ± 2.28 % and was positively correlated with dry weight such that hatchling larvae of greater dry weight had slightly greater carbon content than larvae of lower dry weight; Spearman's rank correlation rho = 0.344, *p* = 4.44e-06 (Figure 4A). Hatchling nitrogen composition (%*N*) ranged from 8.45 to 11.66% with a mean of 9.91 ± 0.58% and was moderately negatively correlated with dry weight; rho = -0.324, *p* = 1.61e-05 (Figure 4B). Hatchling C:N ratio ranged from 3.74 to 5.43 with a mean of 4.65 ± 0.34. As a consequence of the moderate positive relationship between carbon content and dry weight and the moderate negative relationship between nitrogen content and dry weight in hatchling larvae, the ratio of carbon to nitrogen (C:N) increased with increasing dry weight in newly hatched larvae (rho = 0.459, *p* = 321e-10; Figure 4C).

Hatchling nitrogen mass ranged from 9.71 to 16.94 µg ind^{-1} with a mean of 13.18 ± 1.35 µg ind^{-1}. Despite the negative relationship between nitrogen content (%*N*) and larval dry weight (Figure 4B), the mass of nitrogen was positively and strongly correlated with larval dry weight (rho= 0.838, *p* < 2.2e-16) and, therefore, larvae of greater dry weight had considerably more nitrogen mass than larvae of lower dry weight (Figure 4D). Similarly, larvae of greater dry weight had considerably higher carbon mass, as there was a strong relationship between dry weight and carbon mass in newly hatched larvae (r = 0.932, *p* < 2.2e-16) (Figure 4D). Hatchling carbon mass ranged from 41.58 to 85.94 µg ind^{-1} with a mean of 61.31 ± 8.01 µg ind^{-1}. Consequently, some larvae hatched with almost half the carbon mass (and therefore lipid reserves) of other larvae (Figure 4D).

3.4 Per-offspring investment and maternal carapace length

Egg dry weight was weakly, positively correlated with maternal size (rho = 0.131, *p* = 8.289e-06), whilst larval dry weight was positively correlated with maternal CL (rho = 0.478, *p* < 2.2e-16; Figure 5A). Maternal investment per offspring (i.e. egg or larva dry weight (µg) corrected for maternal carapace length (mm)) was negatively correlated with maternal size for both eggs and larvae (Figure 5B). Interestingly, this correlation was stronger for eggs (rho = -0.811, $p < 2.2e\text{-}16$) than it was for larvae (rho = -0.451, $p < 2.2e\text{-}16$; Figure 5B).

3.5 Per-offspring investment and environmental temperature

Correlations were observed between per-offspring investment (both offspring dry weight and maternal investment per offspring) and averaged environmental temperature; however, these relationships were positive or negative depending on the period over which averages were calculated (Figure 6). A weak, negative correlation was identified between egg dry weight and ave. temp., averaged from the start of the winter cold period ($p = 6.61e^{-5}$, rho = -0.118; Figure 6B). Likewise, weak, negative correlations were identified between egg dry weight and ave. T_{min} and ave. temp., averaged from the end of the winter cold period ($p = 1.22e^{-8}$, rho = -0.168; $p = 0.008$, rho = -0.078, respectively); the strongest correlation was with ave. T_{min} (Figure 6G). Negative correlations were also found for the relationship between larval dry weight and ave. T_{min}, ave. temp., and ave. T_{max}, averaged from the start of the winter period ($p < 2.2e^{-16}$, rho = -0.423; $p < 2.2e^{-16}$, rho = -0.295; $p = 1.058e^{-5}$, rho = -0.123, respectively); the strongest of these correlations was with ave. T_{min} (Figure 6A). Conversely, larval dry weight was positively correlated with ave. temp. and ave. T_{max}, averaged from the end of the winter cold period ($p = 2.50\ e^{-7}$, rho = 0.143; $p < 2.2e^{-16}$, rho = 2.601, respectively); the strongest correlation was with ave. T_{max} (Figure 6I).

Maternal investment per offspring was negatively correlated with ave. T_{min} and ave. temp, averaged from the start of the winter cold period, for eggs ($p < 2.2e^{-16}$, rho = -0.301; $p < 2.2e^{-16}$, rho = -0.274, respectively); the strongest correlation was with ave. T_{min} (Figure 6D). For larvae, maternal investment per offspring was negatively correlated with ave. T_{min}, ave.

temp., and ave. T_{max}, averaged from the start of the winter cold period (p <2.2e^{-16}, rho = -0.265; p <2.2e^{-16}, rho = -0.269; p < 1.09e^{-10}, rho = -0.179, respectively); the strongest correlations were with ave. T_{min} and ave. temp. (Figure 6D,E). In contrast, correlations between maternal per offspring investment and environmental temperature averaged from the end of the winter period were all positive (Figure 6). Positive correlations between ave. T_{min}, ave. temp., and ave. T_{max}, averaged from the end of the winter cold period were identified for eggs (p = 1.376e^{-8}, rho = 0.167; p <2.2e^{-16}, rho = 0.395; p <2.2e^{-16}, rho = 0.489, respectively) and larvae (p = 0.113e^{-3}, rho = 0.108; p 1.79e^{-13}, rho = 0.203; p = 6.33e^{-5}, rho = 0.215, respectively); the strongest correlations for both eggs and larvae were with ave. T_{max} (Figure 6L). A strong, positive correlation was also found between GDD,0 and maternal investment per offspring for eggs (, p < 2.2e^{-16}, rho = 0.322) whilst only a very weak correlation was found between GDD,0 and maternal investment per offspring for larvae (p = 0.013, rho = 0.069; Figure 7).

4. DISCUSSION

Here, we report data on reproductive investment (and environmental variability, see supplement) for the palaemonid shrimp, *Palaemon varians*. Although considerable seasonal and inter-annual variation in temperature and salinity were observed, no associated change in per-offspring investment was seen. Instead, a key determinant of per-offspring investment was maternal size. This may indirectly be influenced by environmental conditions during development, but over longer time scales. Alternatively, per-offspring investment may not be influenced by the adult environment as larval development is expected to take place in a different environment; the adjacent marine environment, which is relatively stable. Conditions in the adult environment may not accurately predict those in the larval environment and, therefore, plastic adjustments in per-offspring investment may not be advantageous.

4.2 Maternal size

Maternal size varied across the breeding season for ovigerous *Palaemon varians* brooding newly extruded eggs and hatching larvae. This variation was consistent between years and between ovigerous *P. varians* brooding newly extruded eggs and hatching larvae (Figure 2), suggesting that the change in female size across the breeding season was influenced by population structure. Variations in the size of breeding females have been documented for *Palaemon macrodactylus* Rathbun, 1902; female size within this species decreased across the breeding season (Vázquez et al. 2012). This decrease in female size was a result of large females breeding first, then all mature females breeding, lowering the average size. Large females then died off and a cohort of smaller females joined the breeding population, resulting in a further reduction in the average size of breeding females (Vázquez et al. 2012). Similarly, for two populations of *Palaemon pugio* (Holthuis, 1949), Alon and Stancyk (1982) found two female cohorts and noted variable breeding female size across the breeding season, with female size generally decreasing across the breeding season. Hindley (2001) suggested that within a population of *P. varians* from the Ribble Estuary (Lancashire, UK), large females have a second round of breeding. Data of size frequency presented here, though preliminary, suggests that there are several cohorts of females. The trend in variation in the size of breeding females across the breeding season may be associated with variation in timing of breeding between females of differing cohorts.

4.3 Per-offspring investment

4.3.1 Egg volume as a proxy

Among marine invertebrates generally, but especially within echinoderms, a positive relationship between egg volume and egg energy content and organic dry weight is well documented and has repeatedly been demonstrated for numerous taxa (McEdward & Chai 1991, Clarke 1993, Jaeckle 1995, McEdward & Morgan 2001). This relationship is known at both the inter- and intra-specific level, though at the intraspecific level and within an individual female's brood the correlation is much weaker (McEdward & Chai 1991, Clarke 1993, Jaeckle 1995, Moran and McAlister 2009, McEdward & Morgan 2001). The results presented here demonstrate that within *P. varians,* there is a positive relationship between egg volume and dry weight (Figure 3), consistent with previous literature (e.g. Clarke 1993). The use of egg volume as a proxy for egg energy content has received criticism as, although often a positive relationship exists, egg volume is not an accurate proxy for energy content (Moran & McAlister 2009, Moran et al. 2013). The results presented here for *Palaemon varians* are consistent with this. Although a positive relationship exists and was strong (rho = 0.635), there was considerable scatter of data points. We suggest that egg volume should be used only as a proxy of last resort where other measures (such as dry weight, composition) are not possible.

Variation in egg water content provides a potential explanation as to why egg volume may be a poor proxy for dry weight (Figure 3). Osmotic water uptake under low-salinity conditions is thought the account for egg size differences observed for the estuarine crab *Neohelice granulata* (Dana, 1851) and the amphipod *Gammarus salinus* Spooner, 1947, highlighting that egg water content can vary at the intraspecific level (Gimenez and Anger 2001, Skadsheim 1989, see Moran and McAlister 2009). Whilst egg water content may provide an explanation as to why egg volume varies, it does not offer an explanation as to why egg volume and energy content are decoupled. Moran and McAlister (2009) suggest that for freespawning marine invertebrates, water content increases in egg size provide an increased target size for sperm, but that otherwise egg volume/size is not always an appropriate measure of factors under selection. Whilst egg energy content is likely under strong selective pressure, egg volume may not be and hence these traits may decouple, within limits.

4.3.2 Larval composition

Interestingly, larval composition differed between larvae of differing dry weight (Figure 4). Larval protein content (%N) decreased with increasing larval dry weight whilst lipid content (%C) increased; as such, larger larvae had significantly greater lipid reserves than smaller larvae. At the inter-specific level for seven species of echinoderm, two polychaetes, and an oyster, Strathmann and Vedder (1977) found that smaller diameter eggs had proportionally greater organic matter reserves than larger diameter eggs. In contrast for *P. varians*, larger hatchlings have higher lipid content than smaller hatchlings (Figure 4). Owing to the greater mass of hatchlings with higher dry weight, both protein and lipid mass (nitrogen and carbon, respectively) were higher for hatchlings with greater dry weights (Figure 4). Geister et al. (2009) demonstrated a similar trend for butterfly (*Bicyclus anynana* [Butler, 1879]) eggs produced at different temperatures. Hatchlings from eggs produced at 20 °C had higher absolute amounts of water (+11.1 %), lipids (+20.4 %), and proteins (+35.7 %) than hatchlings from eggs produced at 27 °C (Geister et al. 2009); however, the relative composition of eggs produced at 20 and 27 °C was similar (Geister et al. 2009). In contrast, two populations of the echinoid, *Arbacia lixula* (Linnaeus, 1758), were found to produce eggs that differed in size and lipid and protein content; larger eggs having relatively higher lipid and protein content (George et al. 1990). Similarly, echinoderms maintained under differing food rations produce differing size eggs with differing lipid and protein contents; those provided poor food ratios produced smaller eggs with relatively lower protein and lipid contents (see George 1996). Differences in hatchling dry weight and composition reported here for *P. varians* may indicate differing adult nutritional states. The possibility, however, that such differences arise through conditions during embryogenesis should not be ignored. For example, within the snapping shrimp, *Betaeus emarginatus* (H. Milne-Edwards, 1837), temperature significantly affected larval size at hatching: larvae being smaller after developing at higher temperatures (Wehrtmann & Lopez 2003, see also Smith & Thatje 2013). Similarly, significant differences between embryos within broods may be associated with the position of embryos within the brood; i.e. on different pleopods (e.g. Pochelon et al. 2011). Differences in larval dry weight were strongly correlated with maternal size, but individual females produced larvae of a range of dry weights (Figure 5). Given that the larval lipid (carbon) component increases more than the protein (nitrogen) component as offspring size increases (Figure 4D), larvae of different sizes vary more in their energy reserves than structural component. Pettersen et al. (2015) predict greater metabolic efficiency for larger offspring because of higher energy content relative to structural components compared with smaller offspring. Therefore, larger larvae have greater lipid reserves and may utilise those reserves more efficiently than smaller larvae.

4.3.3 Environmental influence on per-offspring investment

Variations in per-offspring investment are known within populations on temporal scales and have been demonstrated for decapod crustaceans inter-annually (e.g. Kattner et al., 1994), between seasons (e.g. Boddeke, 1982, Sheader 1983, Oh and Hartnoll, 2004, Urzua et al., 2012), and within a single breeding season (e.g. Sampedro et al., 1997, Bas et al., 2007, González-Ortegón et al. 2018). Many of these examples have been associated with temperature differences during gonad development (Kerfoot 1974, Perrin 1988). Data presented here offer a confusing picture of the relationship between environmental temperature and per-offspring investment for *Palaemon varians* (Figure 6). Maternal investment per offspring was negatively correlated with temperature, averaged from the start of the winter cold period until collection date, echoing the temperature-size rule demonstrated for per-offspring investment (e.g. Fischer et al. 2003a,b,c). However, the opposite, positive relationship was observed when temperature was averaged from the end of the winter cold period until collection date. Interestingly, maternal investment per offspring was most strongly correlated with ave. T_{min} when temperature was averaged from the start of the winter cold period, and ave. T_{max} when temperature was averaged from the end of the winter cold period (Figure 6D, L). This raises the possibility that it is the average temperature highs and lows which most greatly influence shrimp physiology. Calculation of GDD,0 revealed a strong correlation with maternal investment per offspring for eggs, but a weak correlation for larvae (Figure 7). These data suggest that as the breeding seasons progresses, *P. varians* experiences warmer temperatures, accumulates more growing degree days, and is able to invest greater resources per offspring in eggs. The strength of correlations between environmental temperature or GDD,0 per-offspring investment was greater for eggs than larvae, suggesting that any influence of temperature may differently affect gonad development and embryogenesis.

P. varians were often observed ‘sunbathing’ in shallows on sunny days and after heavy rainfall shrimp were often less numerous (see Supplementary File 1, section S1.1). Coupled with this is that during the breeding season females were caught less often than males (Figure 1A), suggesting that shrimp likely migrate within ditches to find favourable conditions and this may be especially true of ovigerous shrimp. Indeed, shrimp avoid hypoxia were possible and long-term cyclic hypoxia has been shown to reduce egg dry weight by 24% (Peruzza et al. 2018). The impact of the environment on per-offspring investment may be mediated by maternal behaviour. The correlations observed here do not mean causation, and experimental data are requisite to investigate the effects of temperature on per-offspring investment in *P. varians*.

4.4 Maternal size and per-offspring investment

Among arthropods, female size is known to influence offspring provisioning. In general, larger females give rise to larger offspring, this relationship is often weak and is certainly not ubiquitous; indeed, negative relationships are documented and studies, which found no relationship, may be under reported (Fox & Czesak 2000, Marshall & Keough 2007). Within *Palaemon varians*, egg size is positively, though weakly, correlated to maternal size, similarly, larval size is positively correlated with maternal size (Figure 5). Adaptive explanations have been proposed where correlations between maternal size and offspring size have been identified (Parker & Begon 1986, Fox & Czesak 2000, Sakai & Harada 2001, Marshall & Keough 2007). For example, larger mothers may provision offspring more efficiently than smaller mothers (Sakai & Harada 2001) or larger, more fecund, mothers may better provision offspring to compensate for higher levels of sibling competition (Parker and Begon 1986). Larger female *Palaemon serratus* were found to feed at a higher trophic level than their smaller counterparts and produce eggs with greater carbon and nitrogen content, demonstrating the importance of diet on offspring provisioning (González-Ortegón et al. 2018). Morphological and anatomical scaling may also play a role in the trend, as larger females are able to produce larger offspring (Fox & Czesak 2000). Too little data are presently available to evaluate the merits of the above hypotheses, especially within marine invertebrates (Marshall & Keough 2007). Intriguingly for *P. varians*, our data suggest that the relationship between maternal size and offspring size is greater for larvae than for eggs (Figure 5). Maternal size may have implications for embryonic development; for example, larger females may be better able to ventilate the brood by pleopod flapping or may out-compete smaller females for the best environment in which to brood embryos. However, the small sample size of small females brooding mature embryos may have biased our results and the interpretation that female size influenced embryonic development should be treated with caution. Fox and Csezak (2000) highlighted a trend for larger females to invest a smaller proportion of their resources per offspring. *Palaemon varians* conforms to this trend; both egg and larval dry weight, adjusted for female size, decrease with increasing maternal size (Figure 5). Assuming that the resources available to reproduction increases with maternal size, a decrease in egg and larval dry weight adjusted for female size suggests lower proportions of resource investment per offspring. Therefore, whilst larger females may produce larger offspring, the change is not proportional to the increase in maternal size, suggesting that the production of larger offspring is relatively less costly for larger females. Both the relationship between maternal size and offspring size, and the relationship between maternal size and the proportion of resources invested per offspring raise important questions for the timing of reproduction within semalparous animals such as *P. varians*. It would appear that reproducing as a large female produces better provisioned offspring; therefore, delaying reproduction until a larger size would appear advantageous. Investing energy into reproduction whilst at a smaller size appears disadvantageous. However, delaying reproduction may be costly too as the chances of suffering mortality before reproducing increase. There would, therefore, appear to be a trade-off between the timing of reproduction and the quality of offspring produced and this trade-off would, presumably, differ between environments. It is interesting to note that smaller females bred more in mid-season; could conditions be most favourable at this time, minimising the risk to small offspring? Although we did not measure fecundity, the trade-off between this trait and per-offspring provisioning presumably changes with female size. If investment per offspring increased proportional to female size, larger females would be capable of producing larger eggs than was observed. This suggests that larger females sacrifice some per-offspring investment in favour of increased fecundity, whilst smaller females reduce fecundity in favour of increased per-offspring investment. More data are requisite to draw conclusions about such potential trade-offs.

4.5. Conclusions

Temperature and salinity fluctuated over short time scales as well as long-term seasonal change within Lymington salt marsh. The size of ovigerous female *Palaemon varians* varied across the breeding season, with the average size decreasing in mid-breeding season. This was likely due to population structure and different female cohorts breeding at different times. Egg dry weight and volume were positively correlated for *P. varians*, but the low rho value and considerable scatter indicate that egg volume may be used with only limited accuracy as a proxy for dry weight and per-offspring investment. Larval composition varied with larval dry weight so that larvae of higher initial dry weight had proportionately greater carbon and nitrogen content. Maternal carapace length was a key determinant of per-offspring investment for *P. varians* with larger females able to produce larger eggs and larvae. Together, these data demonstrate variation in egg and larval composition for an aquatic invertebrate. Related to maternal size and the timing of breeding, differences in per-offspring investment will affect early life traits and survivorship, influencing *P. varians* population structure and the wider ecosystem.

Acknowledgements

Andrew Oliphant was supported by a PhD studentship from the University of Southampton.

Compliance with Ethical Standards:

Conflict of Interest: Authors declare no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

Alon NC, Stancyk SE (1982) Variation in life-history patterns of the grass shrimp *Palaemonetes pugio* in two South Carolina estuarine systems. Marine Biology 68:265-276

Anger K (2001) The biology of decapod crustacean larvae. AA, Balkema Lisse

Bas CC, Spivak ED, Anger K (2007) Seasonal and interpopulational variability in fecundity, egg size, and elemental composition (CHN) of eggs and larvae in a grapsoid crab, *Chasmagnathus granulatus*. Helgoland Marine Research 61:225-237

Bayne BL, Holland DL, Moore MN, Lowe DM, Widdows J (1978) Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. Journal of the Marine Biological Association of the UK 58:825-841

Boddeke R (1982) The occurrence of "winter" and "summer" eggs in the brown shrimp (*Crangon crangon*) and the impact on recruitment. Netherlands Journal of Sea Research 16:151-162

Chester CM (1996) The effect of adult nutrition on reproduction and development of the estuarine nudibranch, *Tenellia adspersa* (Nordmann, 1845). Journal of Experimental Marine Biology and Ecology 198:113-130

Clarke A (1993) Egg size and egg composition in polar shrimps (Caridea: Decapoda). Journal of Experimental Marine Biology and Ecology 168:189-203

De Graves S, Ashelby CW (2013) A re-appraisal of the systematic status of selected genera in Palaemoninae (Crustacea: Decapoda: Palaemonidae). Zootaxa 3734:331-344

Fincham AA (1977) Larval development of British prawns and shrimps (Crustacea: Decapoda: Natantia). 1. Laboratory methods and a review of *Palaemon* (*Paleander*) *elegans* Rathke 1837. Bulletin of the British Museum (Natural History) 31:1-28

Fincham AA (1979) Larval development of British prawns and shrimps (Crustacea: Decapoda: Natantia). 2. *Palaemonetes* (*Palaemonetes*) *varians* (Leach, 1814) and morphological variation. Bulletin of the British Museum (Natural History), Zoology Series 35:163-182

Fincham AA (1983) Larval development of British prawns and shrimps (Crustacea: Decapoda: Natantia). 4. *Palaemon (Palaemon) serratus* (Pennant, 1777) and functional morphology of swimming. Bulletin of the British Museum (Natural History), Zoology Series 44:125-161

Fischer K, Bot ANM, Brakefield PM, Zwaan B (2003a) Fitness consequences of temperature-mediated egg size plasticity in a butterfly. Functional Ecology 17:803810

Fischer K, Brakefield PM, Zwaan BJ (2003b) Plasticity in butterfly egg size: why larger offspring at lower temperatures? Ecology 84:3138-3147

Fischer K, Eenhoorn E, Bot ANM, Brakefield PM, Zwaan B (2003c) Cooler butterflies lay larger eggs: devlopmental plasticity versus acclimation. Proceedings of the Royal Society B: Biological Sciences 270:2051-2056

Fischer S, Thatje S, Brey T (2009) Early egg traits in *Cancer setosus* (Decapoda, Brachyura): effects of temperature and female size. Marine Ecology Progress Series 377:193-202

Fox CW, Czesak ME (2000) Evolutionary ecology of progeny size in arthropods. Annual Review of Entomology 45:341-369

Geister TL, Lorenz MW, Hoffmann KH, Fischer K (2009) Energetics of embryonic development: effects of temperature on egg and hatchling composition in a butterfly. Journal of Comparative Physiology B 179:87-98

George SB (1990) Population and seasonal differences in egg quality of *Arbacia lixula* (Echinodermate: Echinoidea). Invertebrate Reproduction & Development 17:111-121

George SB (1996) Echinoderm egg and larval quality as a function of adult nutritional state. Oceanologica Acta 19:297-308

George SB, Cellario C, Fenaux L (1990) Population differences in egg quality of *Arbacia lixula* (Echinodermata: Echinoidea): proximate composition of eggs and larval development. Journal of Experimental Marine Biology and Ecology 141:107-118

Giménez L (2006) Phenotypic links in complex life cycles: conclusions from studies with decapod crustaceans. Integrative and Comparative Biology 46:615-622

Giménez L, Anger K, Torres G (2004) Linking life history traits in successive phases of a complex life cycle: effects of larval biomass on early juvenile development in an estuarine crab, *Chasmagnathus granulata*. Oikos 104:570-580

Giménez L, Torres G (2002) Larval growth in the estuarine crab *Chasmagnathus granulata*: the importance of salinity experience during embryonic development, and the initial larval biomass. Marine Biology 141:877-885

González-Ortegón E, and Giménez L (2014) Environmentally mediated phenotypic links and performance in larvae of a marine invertebrate. Marine Ecology Progress Series 502:185-195

González-Ortegón E, Vay LL, Walton MEM, Giménez L (2018) Maternal Trophic Status and Offpsring Phenotype in a Marine Invertebrate. Scientific Reports 8:9618 https://doi.org/10.1038/s41598-018-27709-2

Hindley J (2001) The ecology and dynamics of the brackish water prawn, *Palaemonetes varians* (Leach) and its interrelationship with the common goby, *Pomatoschistus microps* (Krøyer) in artificial coastal lagoons of the Ribble Estuary, Lancashire. Doctor of Philosophy, University of Lancaster, Edge Hill

Jaeckle WB (1995) Variation in the size, energy content, and biochemical composition of invertebrate eggs: correlates to the mode of larval development. In: McEdward LR (ed) Ecology of Marine Invertebrate Larvae. CRC Press Boca, London

Kattner G, Wehrtmann IS, Merck T (1994) Interannual variations of lipids and fatty acids during larval development of *Crangon* spp. in the German Bight, North Sea. Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology 107:103-110

Kerfoot WC (1974) Egg-size cycle of a cladoceran. Ecology 55:1259-1270

Lardies MA, Castilla JC (2001) Latitudinal variation in the reproductive biology of the commensal crab *Pinnaxodes chilensis* (Decapoda: Pinnotheridae) along the Chilean coast. Marine Biology 139:1125-1133

Lardies MA, Wehrtmann IS (2001) Latitudinal variation in the reproductive biology of *Betaeus truncatus* (Decapoda: Alpheidae) along the Chilean coast. Ophelia 55:5567

Liefting M, Weerenbeck M, van Dooremalen C, Ellers J (2010) Temperature-induced plasticity in egg size and resistance of eggs to temperature stress in a soil arthropod. Functional Ecology 24:1291-1298

Linck B (1995) Einfluß von Temperatur und Salzgehalt auf die Larven der Nordseegarnele *Crangon crangon*. University of Oldenburg, Germany, MSc thesis

Marshall D, Keough MJ (2007) The evolutionary ecology of offspring size in marine invertebrates. Advances in Marine Biology 53:1-60

McEdward LR, Chai F-S (1991) Size and energy content of eggs from echinoderms with pelagic lecithotrophic development. Journal of Experimental Marine Biology and Ecology 147:95-102

McEdward LR, Morgan KH (2001) Interspecific relationships between egg size and the level of parental investment per offspring in echinoderms. Biological Bulletin 200:33-50

Moran AL, McAlister JS (2009) Egg size as a life history character of marine invertebrates: Is it all it's cracked up to be? Biological Bulletin 216:226-242

Moran AL, McAlister JS, Whitehill EAG (2013) Eggs as Energy: Revisiting the scaling of egg size and energetic content among echinoderms. Biological Bulletin 224:184191

Morris JP, Thatje S, Cottin D, Oliphant A, Brown A, Shillito B, Ravaux J, Hauton C (2015) The potential for climate-driven bathymetric range shift: sustained temperature and pressure exposures on a marine ectotherm, *Palaemonetes varians*. Royal Society Open Science 2:150472

Müller Y, Ammar D, Nazari E (2004) Embryonic development of four species of palaemonid prawns (Crustacea, Decapoda): pre-naupliar, naupliar and post-naupliar periods. Revista Brasileira de Zoologia 21:27-32

Oh CW, Hartnoll RG (2004) Reproductive biology of the common shrimp *Crangon crangon* (Decapoda: Crangonidae) in the central Irish Sea. Marine Biology 144:303-316

Oliphant A, Thatje S, Brown A, Morini M, Ravaux J, Shillito B (2011) Pressure tolerance of the shallow-water caridean shrimp *Palaemonetes varians* across its thermal tolerance window. Journal of Experimental Biology 214:1109-1117

Oliphant A, Hauton C, Thatje S, (2013) The implications of temperature-mediated plasticity in larval instar number for development within a marine invertebrate, the shrimp *Palaemonetes varians*. PLoS ONE 8(9)e75785

Oliphant A, Thatje S (2013) Per offspring investment implications for crustacean larval development: evolutionary insights into endotrophy and abbreviated development. Marine Ecology Progress Series 493:207-217

Oliphant A, Thatje S (2014) Energetic adaptations to larval export within the brackish living palaemonine shrimp *Palaemonetes varians*. Marine Ecology Progress Series 505:177-191

Oliphant A, Ichino MC, Thatje S (2014) The influence of per offspring investment (POI) and starvation on larval developmental plasticity within the palaemonid shrimp, *Palaemonetes varians*. Marine Biology 161:2069-2077

Parker GA, Begon M (1986) Optimal egg size and clutch size – effects of environment and maternal phenotype. American Naturalist 128:573-592

Pettersen AK, White CR, Marshall DJ (2015) Why does offspring size affect performance? Integrating metabolic scaling with life-history theory. Proceeding of the Royal Society B Biological Sciences 282:20151946

Perrin N (1988) Why are offspring born larger when it is colder? Phenotypic plasticity for offspring size in the cladoceran *Simocephalus vetulus* (Muller). Functional Ecology 2:283-288

Peruzza L, Gerdol M, Oliphant A, Wilcockson DC, Pallavicini A, Hawkins L, Thatje S, Hauton C (2018) The consequences of daily hypoxia on a European grass shrimp: From short-term responses to long-term effects. Functional Ecology 32:2333-2344

Pochelon PN, da Silva TL, Reis A, Dos Santos A, Queiroga H, Calado R (2011) Interindividual and within-brood variability in the fatty acid profiles of Norway lobster, *Nephrops norvegicus* (L.) embryos. Marine Biology 158:2825-2833

Qian PY, Chia FS(1991) Fecundity and egg size are mediated by food quality in the polychaete worm *Capitella* sp. Journal of Experimental Marine Biology and Ecology 148:11-25

Qian PY (1994) Effect of food quantity on growth and reproductive characteristics of *Capitella* sp. (Annelida: Polychaete). Invertebrate Reproduction and Development 26:175-185

Sampedro MP, Fernández L, Freire J, González-Gurriarán E (1997) Fecundity and reproductive output of *Pisidia longicornis* (Decapoda, Anomura) in the Ría de Arousa (Galicia, NW Spain). Crustaceana 70:95-110

Sheader M (1983) The reproductive biology and ecology of *Gammarus duebeni* (Crustacea: Amphipoda) in southern England. Journal of the Marine Biological Association of the UK 63:517-540

Smith KE, Thatje S (2013) The subtle intracapsular survival of the fittest: maternal investment, sibling conflict, or environmental effects? Ecology 94:2263-2274

Strathmann RR, Vedder K (1977) Size and organic content of eggs of echinoderms and other invertebrates as related to developmental strategies and egg eating. Marine Biology 39:305-309

Urzúa Á, Anger K (2013) Seasonal variations in larval biomass and biochemical composition of brown shrimp, *Crangon crangon* (Decapoda, Caridea), at hatching. Helgoland Marine Research 67:267-277

Urzúa Á, Paschke K, Gebauer P, Anger K (2012) Seasonal and interannual variations in size, biomass and chemical composition of the eggs of the North Sea shrimp, *Crangon crangon* (Decapoda: Caridea). Marine Biology 159:583-599

Vázquez M, Bas CC, Spivak ED (2012) Life history traits of the invasive estuarine shrimp *Palaemon macrodactylus* (Caridea: Palaemonidae) in a marine environment (Mar del Plata, Argentina). Scientia Marina 76:507-516

Wehrtmann IS, Lopez GA (2003) Effects of temperature on the embryonic development and hatchling size of *Betaeus emarginatus* (Decapoda: Caridea: Alpheidae). Journal of Natural History 37:2165-2178

Figures

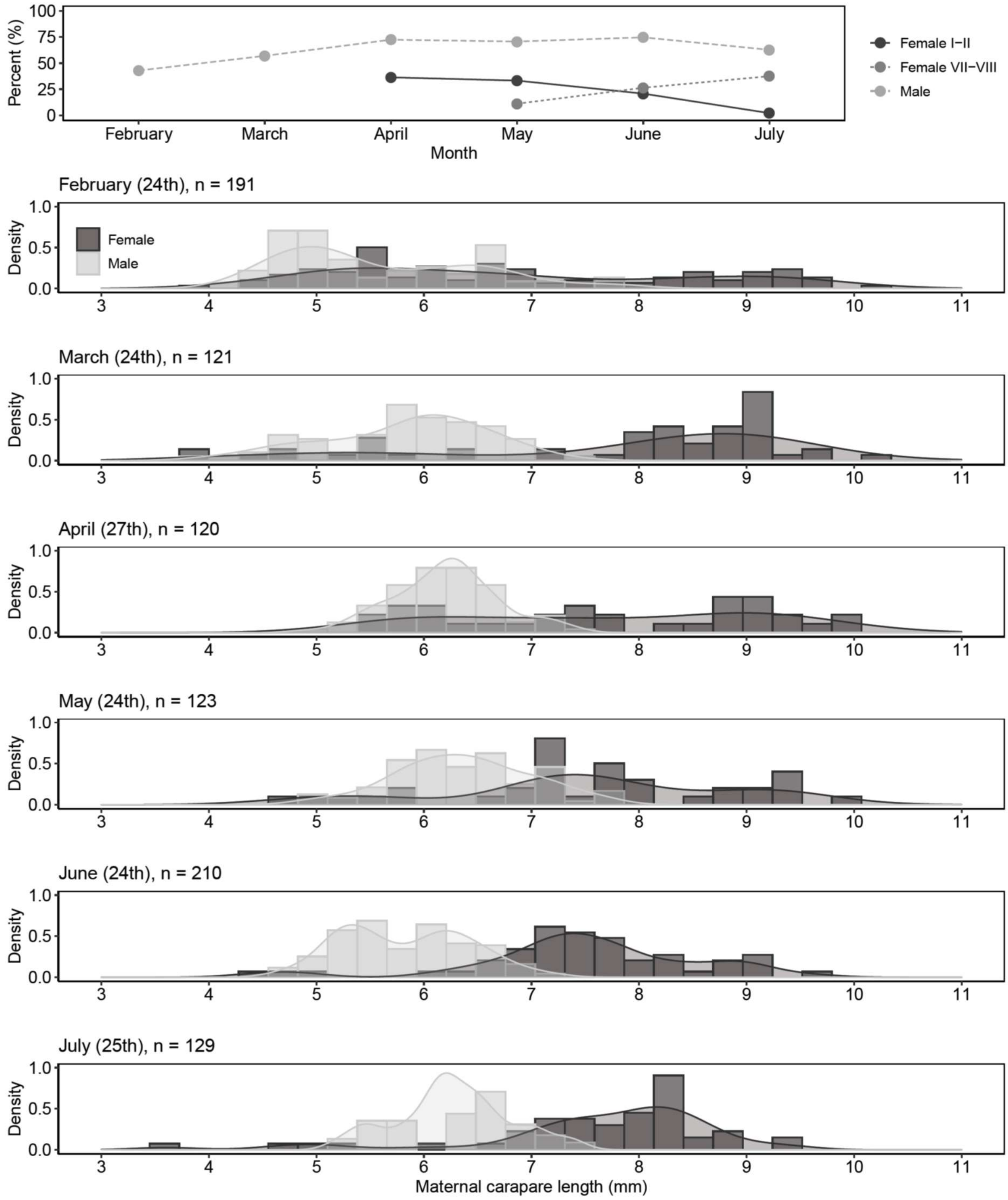

Figure 1. Males percent of total shrimp and females with stage I-II eggs and stage VII-VIII embryos percent of total females in monthly samples. Size frequency density plots for monthly samples of *Palaemon varians* across the 2011 breeding season in Lymington salt marsh (Hampshire, UK).

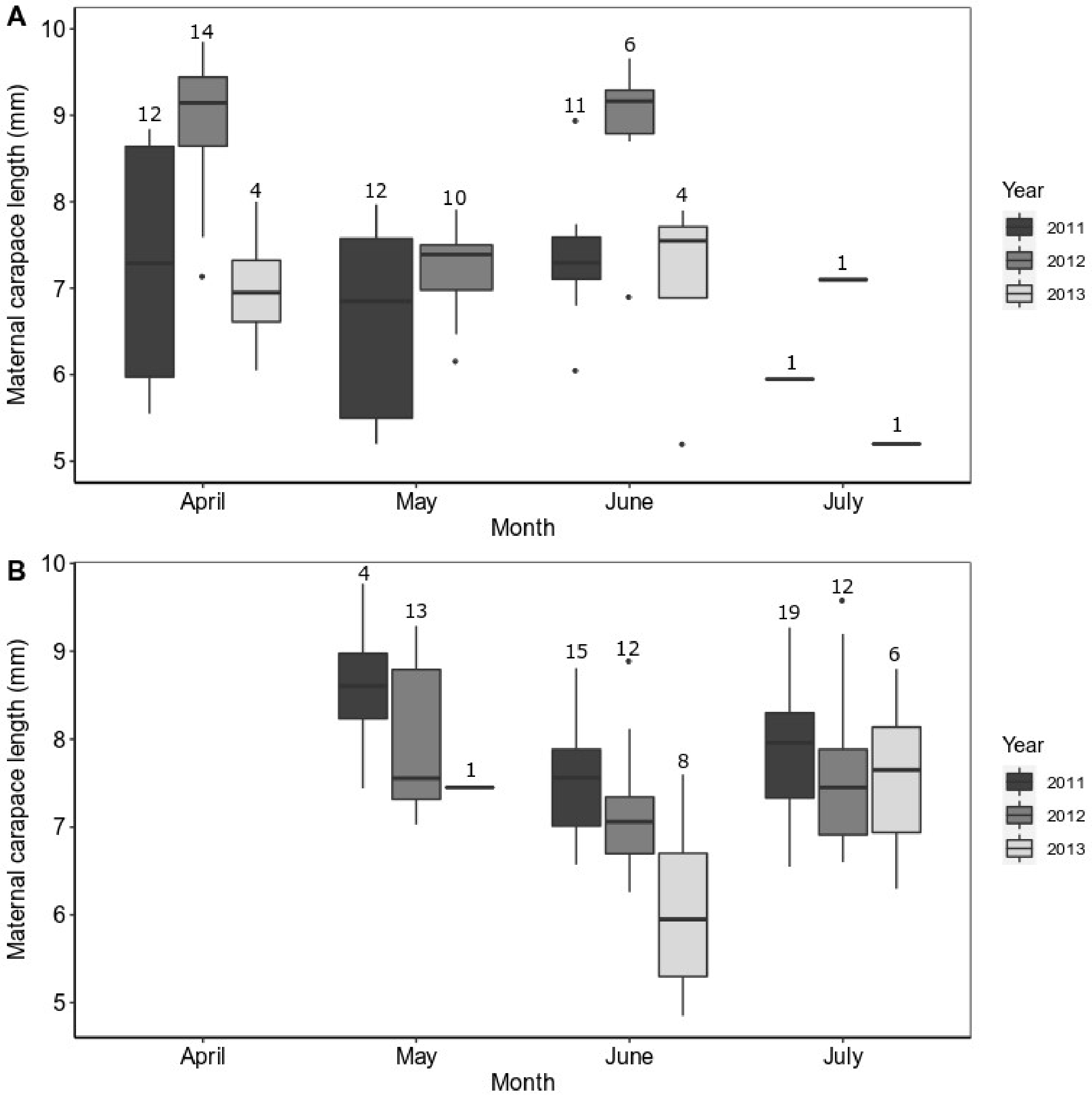

Figure 2. Maternal post-orbital carapace lengths (mm) for females bearing stage I-II eggs (**A**) and stage VII-VIII embryos (**B**) across the breeding season for three consecutive years. *n* are given above each box.

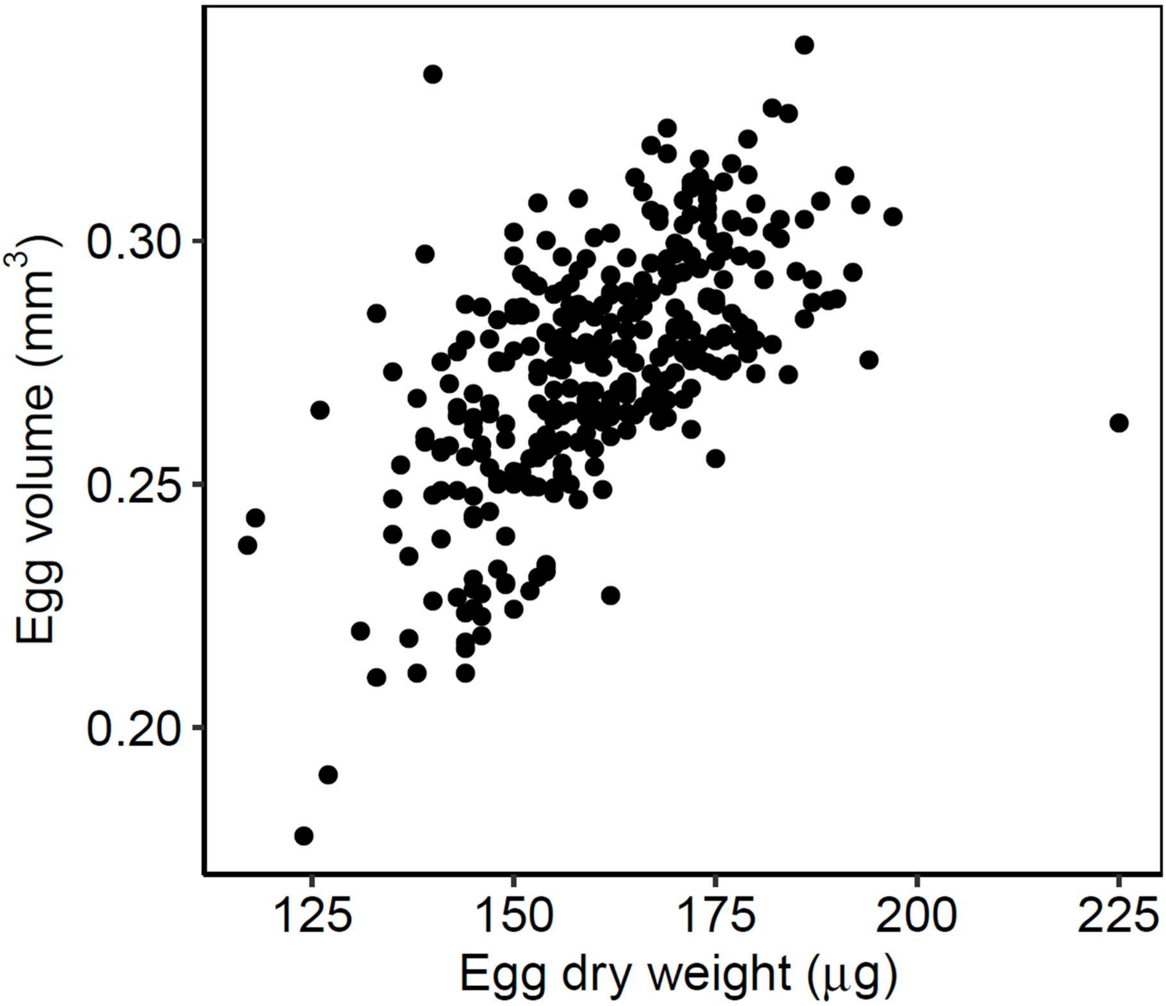

Figure 3. Relationship between egg volume (mm^3) and egg dry weight (μg) for *Palaemon varians* eggs sampled in 2011 from Lymington salt marsh (Hampshire, UK).

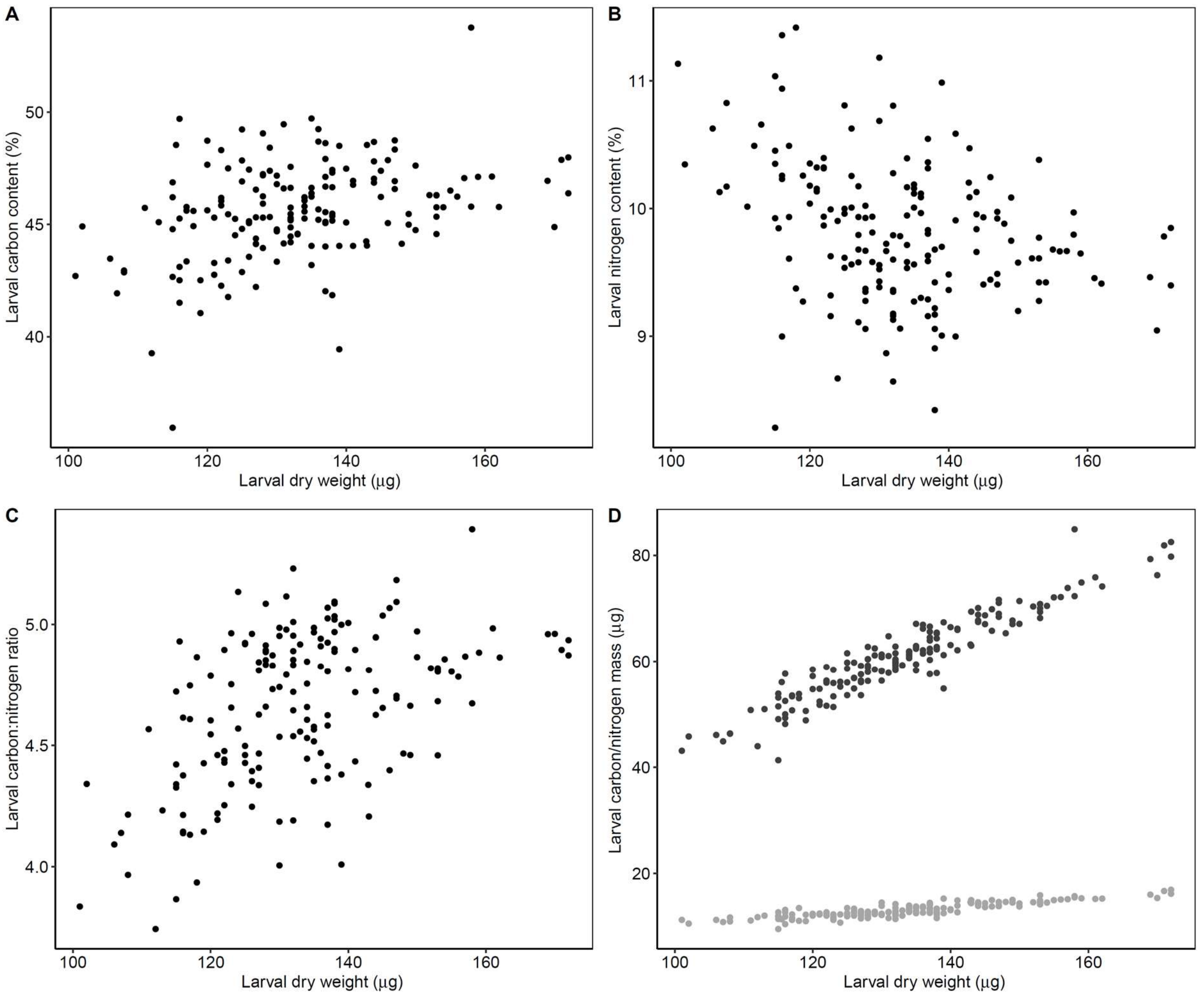

Figure 4. Relationship between larval dry weight and (**A**) carbon content (**B**) nitrogen content (**C**) carbon:nitrogen ratio (**D**) and carbon (dark grey) and nitrogen (light grey) mass for *Palaemon varians* larvae sampled in May and June of 2012 from Lymington salt marsh (Hampshire, UK)

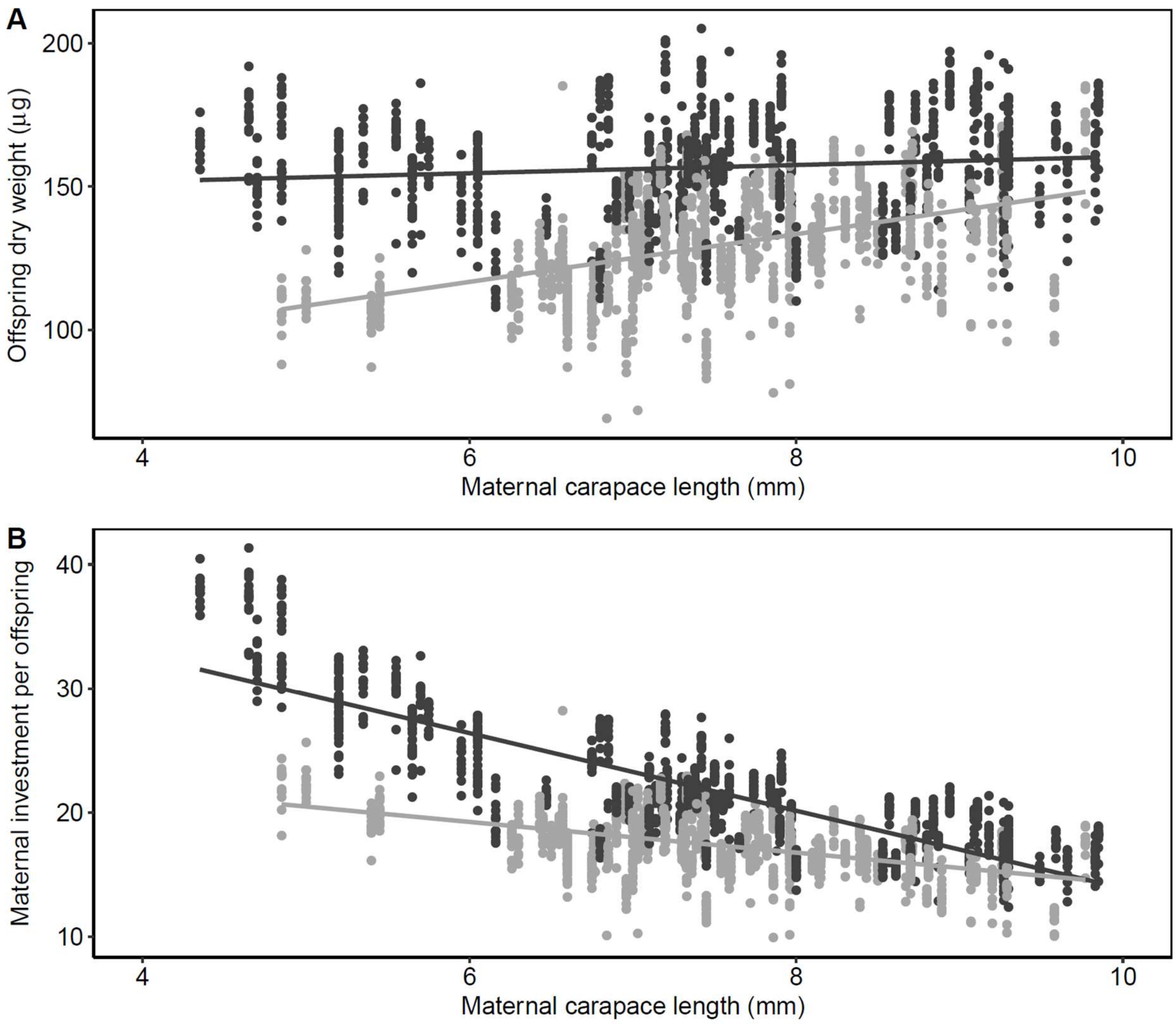

Figure 5. Relationship between maternal post-orbital carapace length (**A)** egg dry weight and larval dry weight (**B)** maternal investment per offspring in eggs and larvae (offspring dry weight adjusted for maternal carapace length; µg/mm). Egg = dark grey, larvae = light grey.

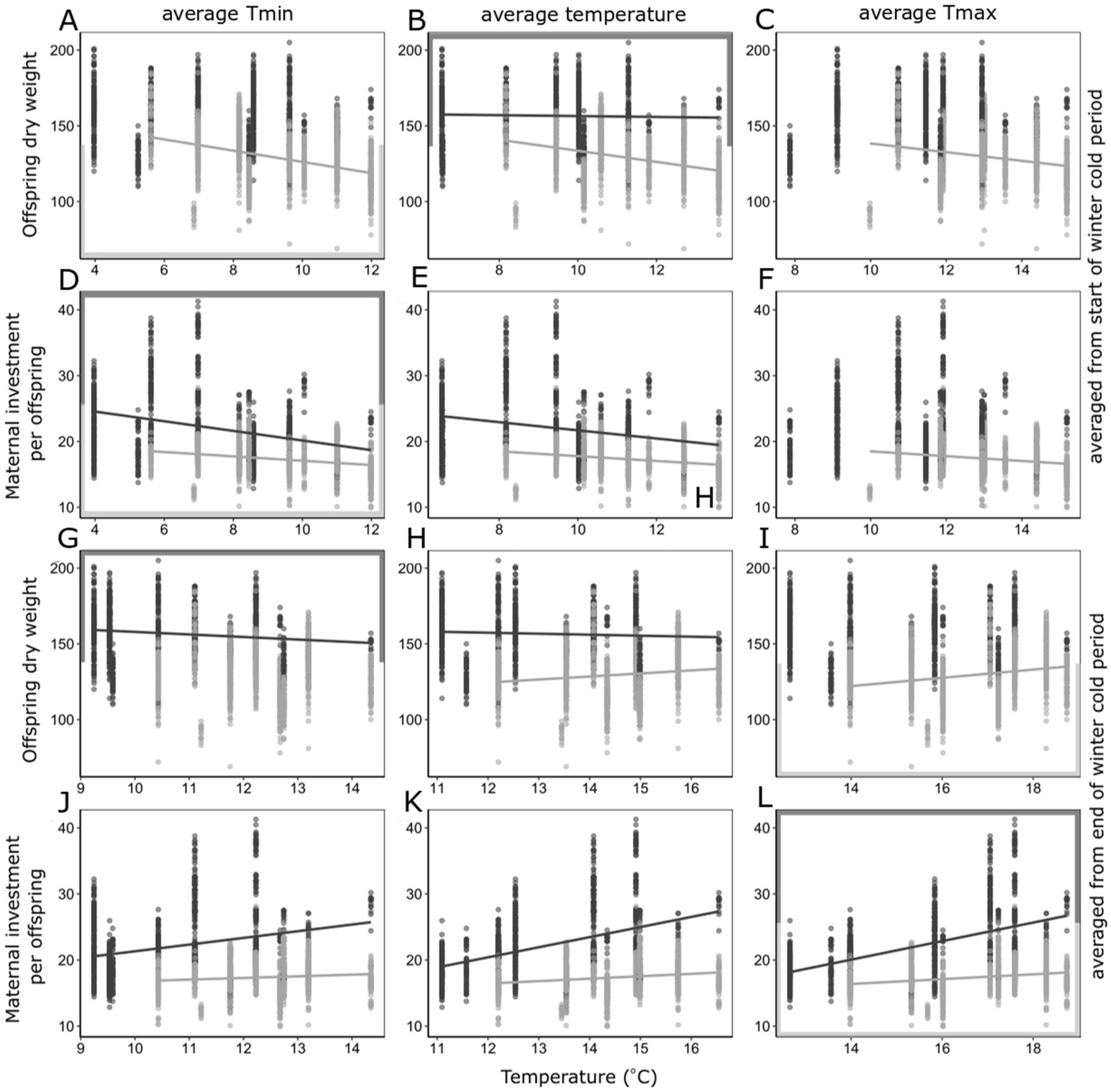

Figure 6. Relationships between offspring dry weight (µg) or maternal investment per offspring (offspring dry weight adjusted for maternal post-orbital carapace length; µg/mm) and daily temperature minimum (ave. T_{min}), daily temperature (ave. temp.), or daily temperature maximum (ave. T_{max}), averaged from the start or end of the winter cold period until collection date. Data points: egg = dark grey, larvae = light grey. Lines of best fit (linear model) match data point colour and are displayed for statistically significant correlations (see

text for *p*-values). The strongest correlations are highlighted (eggs = dark grey, larvae = light grey).

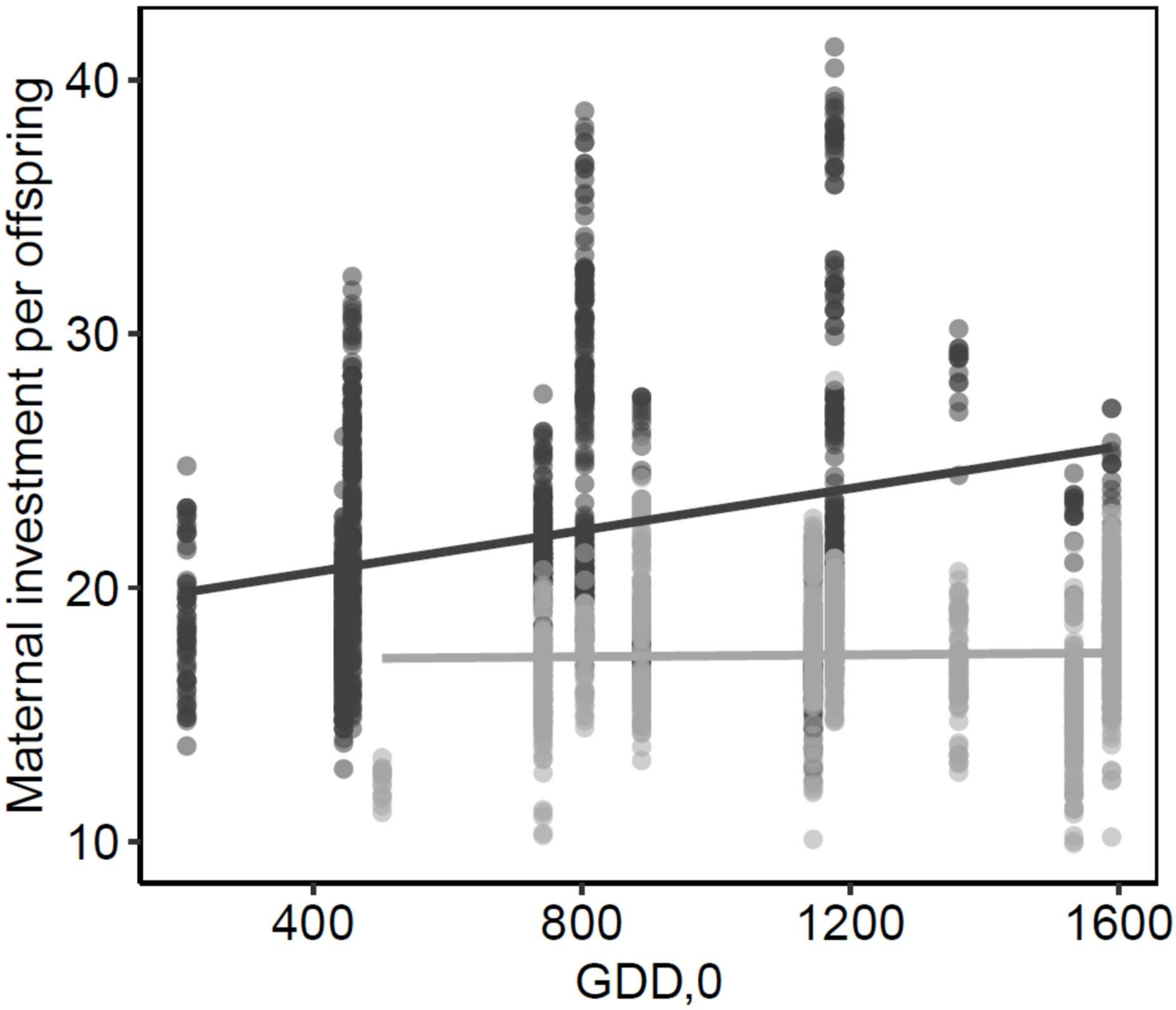

Figure 7. Relationship maternal investment per offspring (offspring dry weight adjusted for maternal post-orbital carapace length; µg/mm) and growing degree days, 0 (GDD,0) for eggs and larvae. Data points: egg = dark grey, larvae = light grey. Lines of best fit (linear model) match data point colour.